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Common Variation in the *NOS1AP* Gene Is Associated With Drug-Induced QT Prolongation and Ventricular Arrhythmia

Yalda Jamshidi, PhD,* Ilja M. Nolte, PhD,† Chrysoula Dalageorgou, BSc,* Dongling Zheng, PhD,* Toby Johnson, PhD,‡ Rachel Bastiaenen, MBBS,§ Suzanne Ruddy, PhD,* Daniel Talbott, BSc,* Kris J. Norris, RN,‖ Harold Snieder, PhD,† Alfred L. George, MD,‖ Vanessa Marshall, MBBS,¶ Saad Shakir, MD,¶ Prince J. Kannankeril, MD,# Patricia B. Munroe, PhD,‡ A. John Camm, MD,§ Steve Jeffery, PhD,* Dan M. Roden, MD,¶ Elijah R. Behr, MBBS, MD§

London and Southampton, United Kingdom; Groningen, the Netherlands; and Nashville, Tennessee

Objectives This study sought to determine whether variations in *NOS1AP* affect drug-induced long QT syndrome (LQTS).

Background Use of antiarrhythmic drugs is limited by the high incidence of serious adverse events including QT prolongation and torsades de pointes. *NOS1AP* gene variants play a role in modulating QT intervals in healthy subjects and severity of presentation in LQTS.

Methods This study carried out an association study using 167 single nucleotide polymorphisms (SNP) spanning the *NOS1AP* gene in 58 Caucasian patients experiencing drug-induced LQTS (dLQTS) and 87 Caucasian controls from the DARE (Drug-Induced Arrhythmia Risk Evaluation) study.

Results The rs10800397 SNP was significantly associated with dLQTS (odds ratio [OR]: 3.3, 99.95% confidence interval [CI]: 1.0 to 10.8, $p = 3.7 \times 10^{-4}$). The associations were more pronounced in the subgroup of amiodarone users, in which 3 SNPs, including rs10800397, were significantly associated (most significant SNP: rs10919035: OR: 5.5, 99.95% CI: 1.1 to 27.9, $p = 3.0 \times 10^{-4}$). We genotyped rs10919035 in an independent replication cohort of 28 amiodarone dLQTS cases versus 173 control subjects (meta-analysis of both studies: OR: 2.81, 99.95% CI: 1.62 to 4.89, $p = 2.4 \times 10^{-4}$). Analysis of corrected QT interval among 74 control subjects from our dataset showed a similar pattern of significance over the gene region as the case-control analysis. This pattern was confirmed in 1,480 control subjects from the BRIGHT (British Genetics of Hypertension Study) cohort (top SNP from DARE: rs12734991 in meta-analysis: increase in corrected QT interval per C allele: 9.1 ± 3.2 ms, $p = 1.7 \times 10^{-4}$).

Conclusions These results provide the first demonstration that common variations in the *NOS1AP* gene are associated with a significant increase in the risk of dLQTS. This study suggests that common variations in the *NOS1AP* gene may have relevance for future pharmacogenomic applications in clinical practice permitting safer prescription of drugs for vulnerable patients. (J Am Coll Cardiol 2012;60:841–50) © 2012 by the American College of Cardiology Foundation

From the *Human Genetics Research Centre, St. George's University of London, London, United Kingdom; †Department of Epidemiology, University of Groningen, University Medical Center Groningen, Groningen, the Netherlands; ‡Clinical Pharmacology and Barts and The London Genome Centre, William Harvey Research Institute, Barts and The London School of Medicine and Dentistry, Queen Mary University of London, Charterhouse Square, London, United Kingdom; §Division of Cardiovascular Sciences, St. George's University of London, London, United Kingdom; ‖Department of Medicine and Pharmacology, Vanderbilt University School of Medicine, Nashville, Tennessee; ¶Drug Safety Research Unit, Southampton, United Kingdom; and the #Department of Pediatrics, Vanderbilt University School of Medicine, Nashville, Tennessee. The DARE study was funded by the British Heart Foundation (BHF), Project Grant No. 06/094 (to Dr. Jamshidi) and Special Program Grant No. SP/02/001 (to Dr. Camm). The Vanderbilt study was supported by Grant No. U01/U19 HL65962, the Pharmacogenomics of Arrhythmia Therapy site of the Pharmacogenomics Research Network, and by a grant from the Fondation Leducq (Trans-Atlantic Network of Excellence "Alliance Against Sudden Cardiac Death," 05 CVD 01). The BRIGHT study was supported by the Medical Research Council of Great Britain (Grant No. G9521010D) and by BHF Grant No. PG/02/128. Genotyping for the Human_CVD BeadChip was supported by BHF Grant No. PG/07/131/24254 (to Dr. Munroe). This work forms part of the research themes contributing to the translational research portfolio for Barts and the London Cardiovascular Biomedical Research Unit, which is supported and funded by the National Institute for Health Research. Dr. Johnson has received research funding from Wellcome Trust (Grant No. 093078/Z/10/Z); and is an employee of GlaxoSmithKline. Dr. George has received research grants from Gilead Sciences and Allergan Inc. Dr. Camm is an advisor and speaker for Boehringer Ingelheim, Bayer, Daiichi, Pfizer, Bristol-Myers Squibb, Sanofi, Servier, Xention, Medtronic, Boston

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Abbreviations and Acronyms

CEU = western and northern European ancestry
CI = confidence interval
DNA = deoxyribonucleic acid
ECG = electrocardiogram
IRB = Institutional Review Board
LD = linkage disequilibrium
LQTS = long QT syndrome
nNOS = neuronal nitric oxide synthase
NOS1AP = nitric oxide synthase 1 adaptor protein
OR = odds ratio
SNP = single nucleotide polymorphism
TdP = torsades de pointes

Currently amiodarone is the most commonly used antiarrhythmic drug, followed closely by sotalol. However, use of antiarrhythmic drugs is limited by the high incidence of bradycardia and QT prolongation, which can result in torsades de pointes (TdP) (1–3). Amiodarone is a class III antiarrhythmic agent inhibiting I_{Kr} (4,5) and increasing action potential duration and the effective refractory period (6,7) (seen as QT prolongation on the surface electrocardiogram [ECG]). Amiodarone also decreases conduction velocity by blocking Na^+ channels (class I effect), reduces the number of beta-adrenergic receptors with a resultant antiadrenergic effect (class II effect), and suppresses Ca^{2+}-mediated action potentials by blocking L-type calcium channels (class IV effect). Sotalol meanwhile is an antiarrhythmic drug with class II and class III properties (5).

It has been suggested that every individual has a physiological "cardiac repolarization reserve" (8), which may be genetically determined and which compensates for any endogenous or exogenous factors (e.g., drugs) that would either decrease repolarizing or increase depolarizing currents during the action potential. It is likely, therefore, that individuals with reduced repolarization reserve are more vulnerable to developing QT-interval prolongation and TdP when exposed to potassium channel–blocking drugs such as amiodarone and sotalol.

Genome-wide analysis has consistently associated common variants of the nitric oxide synthase 1 adaptor protein (*NOS1AP*) gene with QT interval across independent replication studies (9–12). Despite attempts to identify and validate a single functional variant in *NOS1AP* associated with QT interval, resequencing of all exons in *NOS1AP* has not yet identified any missense mutations that explain these results, suggesting that the functional variants associated with these single nucleotide polymorphisms (SNP) are likely to be regulatory in nature (9).

NOS1AP is a regulator of neuronal nitric oxide synthase (nNOS encoded by *NOS1*), an isoform of NOS, which regulates intracellular calcium levels and myocyte contraction in the heart (13–15). The NOS1AP interacts with nNOS to accelerate cardiac repolarization by inhibition of L-type calcium channels (16–19), thereby providing a rationale for the association of *NOS1AP* gene variants with QT-interval duration.

The variability of drug action in individuals can arise because of variation in genes encoding drug targets, genes modulating the overall activity of the complex biological systems within which the drugs act, and genes that are responsible for drug metabolism and elimination. In view of the role of NOS1AP in cardiac repolarization, we hypothesized that genetic variation in the *NOS1AP* gene influences the incidence of drug-induced ventricular arrhythmia and QT prolongation.

Methods

The DARE (Drug-Induced Arrhythmia Risk Evaluation) study is a national cohort of 112 patients experiencing drug-induced ventricular arrhythmias and/or severe QT-interval prolongation in the United Kingdom. A case-control study was established from the DARE study consisting of 59 Caucasian case subjects who had experienced an arrhythmic event associated with drug-induced QT prolongation, and 91 control subjects, all of whom had provided deoxyribonucleic acid (DNA) samples. Ethnicity was self-reported as Caucasian for all cases and controls.

Cases were included if they had 1 or more of the following diagnosed as secondary to a medication: documented classical TdP defined as 3 beats or more of polymorphic ventricular tachycardia associated with QT prolongation and pauses prior to onset of the arrhythmic event; ventricular fibrillation and/or cardiac arrest associated with corrected (QTc)-interval prolongation; and QTc-interval prolongation with a history consistent with cardiac syncope, excluding vasovagal syncope and seizures. After withdrawal of the culpable drug, cessation of ventricular arrhythmia and syncope and at least partial resolution of QT prolongation were required. All QTc intervals were corrected using Bazett's formula and values >450 ms (men) or >470 ms (women) were considered prolonged. Cases were excluded if DNA was unavailable and/or arrhythmias were not documented and/or QT prolongation was absent.

Healthy control subjects were provided from primary care physicians responsible for the cases to ensure geographical matching. Inclusion criteria were no history of drug-induced arrhythmias, ventricular arrhythmias, or the congenital long QT syndrome (LQTS). Control subjects with abnormal resting 12-lead ECGs were excluded.

Clinical and ECG assessment. The case subjects' acute presentation with arrhythmia and/or syncope and past medical history were assessed by obtaining hospital records, interview, and patient questionnaires. The QT and RR

Scientific, Gilead, and Menarini; is a data and safety monitoring board member for Cameron Health, Biotronik, Novartis, Astellas, Forest Labs, Servier, and Bioctcontrol; is an events committee member for Novartis; has research contracts with Sanofi, Boehringer Ingelheim, Daiichi, Menarini, Richmond Pharmacology; and is a consultant for Cardialysis. Dr. Roden has received consulting fees from Astellas, Sanofi, and Warner-Chicott. Dr. Behr has received research funding from Biotronik, the International Serious Adverse Events Consortium, and St. Jude Medical. All other authors have reported that they have no relationships relevant to the contents of this paper to disclose. Drs. Jamshidi and Nolte contributed equally to this paper.

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intervals were measured manually from paper ECGs at stable heart rates by averaging them for up to 5 cardiac cycles (up to 10 cardiac cycles in atrial fibrillation) and Bazett's formula was used to calculate the heart rate corrected QTc interval.

Case subjects, with drug exposure removed, and control subjects underwent resting 500-Hz digital 12-lead ECGs acquired using PC-based Cardionavigator recorders (Del Mar Reynolds, Spacelabs Healthcare, Issaquah, Washington). Each automatically calculated QT interval was checked manually. If they were similar, the automatic measurement was not revised. If not, then the same method described for manual measurements was used.

***NOS1AP* sequencing.** Gene sequencing of *NOS1AP* (ENSG00000198929) exons and intron/exon boundaries was carried out for all case subjects and control subjects using the ABI3130 System (Applied Biosystems, The Medical Biomics Centre, St. George's University London [SGUL], London, United Kingdom) (primers available on request).

NOS1AP Association Study

One hundred and ninety-eight tagging SNPs, derived from the National Center for Biotechnology Information's build 35 of the *NOS1AP* gene, were genotyped in all case subjects and control subjects using the Infinium Human_CVD 50K Bead Array (Illumina-IBC/CVD, Illumina Inc., San Diego, California) (20) and were analyzed using the Illumina platform 500GX (Medical Biomics Centre, SGUL). Seven SNP covered up to 4.3 kb of the upstream region of NOS1AP from the start site of the gene, and after resequencing, we found 2 more SNP, which we added to the dataset. The downstream region of the gene was not covered. Two outliers (1 case subject and 1 control subject) were excluded from the analysis as they had >10% missing data. We checked for ethnic outliers using the complete genetic dataset of the CVD chip. Multidimensional scaling analysis in PLINK (version 1.07, Shaun Purcell, Center for Human Genetic Research, Boston, Massachusetts) (21,22) revealed that 3 control subjects were not of Caucasian descent despite their self-reported Caucasian ethnicity. After these exclusions, there were 58 cases and 87 controls available for analysis. The average call rate of the remaining subjects was 99.9%. Thirty-three SNPs were nonpolymorphic, hence 167 SNPs could be analyzed for association with QT-interval prolongation and drug-induced ventricular arrhythmia.

***NOS1AP* rs10919035 replication cohort.** CASES. For validation, an independent set of 28 amiodarone-treated patients collected at Vanderbilt University Medical Center, under appropriate Institutional Review Board (IRB)-approved protocols was used. Patients were of European descent from North America with drug-induced LQTS, defined as documented TdP associated with reversible QT prolongation during treatment with amiodarone. Covariates included age, sex, self-reported ethnicity, hypokalemia, and the culprit drug at the time of the index arrhythmia.

REPLICATION DRUG-EXPOSED CONTROLS. One hundred and five self-identified European ancestry subjects derived from a clinical study at Vanderbilt University Medical Center, under an IRB-approved protocol were included as drug-exposed control subjects. The study uses electronic medical record–based surveillance to identify patients in whom assorted QT prolonging antiarrhythmics were being initiated.

REPLICATION NORMAL CONTROLS. Sixty-eight self-identified European ancestry subjects derived from a clinical study at Vanderbilt University Medical Center, under an IRB-approved protocol were included as drug-exposed control subjects. Control subjects were normal, healthy volunteers recruited from the general population and challenged with an antiarrhythmic drug (ibutilide) (23).

For this study, control subjects were defined as having the absence of qualifying arrhythmias, <50 ms increase in QTc (by Bazett's formula) interval on drug exposure, and no QTc interval exceeding 500 ms during drug treatment or ibutilide challenge.

The frequency distributions of the 2 control samples were similar, and hence the control data were pooled for analysis.

QTc replication cohort. BRIGHT. Over 2,000 unrelated white European hypertensive individuals from the BRIGHT (British Genetics of Hypertension Study) study (24) were genotyped using the Human_CVD BeadChip (Illumina). Of those, 1,909 individuals passed quality control checks (samples with low call rate, cryptic duplicates and relatives, outliers in ancestry principle component analysis, sex X chromosome mismatch were excluded). Of the 1,909, 1,628 individuals had 12-lead ECG recordings (Siemens-Sicard440, Siemens, Berlin, Germany) (25). For this analysis, we excluded individuals with QRS duration >120 ms (n = 83), and individuals with atrial fibrillation or persistent flutter (Minnesota codes 8-3-1 or 8-3-2; n = 25). No data were available for antiarrhythmic drug consumption. We also excluded individuals with a missing covariate (age; n = 40). Thus, data on 1,480 individuals were tested for association. We used a normal linear model with QTc as outcome and sex, age, and 10 ancestry principal components as covariates to control for population stratification. We analyzed all SNPs within 50 kb of the *NOS1AP* transcript, specifically from rs4657139 (chr1:160296531) to rs457879 (chr1:160602678) inclusive. We excluded SNPs that could not be called with high confidence (4 SNPs) and 1 SNP with a call rate below 98%. The results for 195 SNPs were provided.

Statistical analysis. Case subjects and control subjects from the DARE study were compared for population characteristics using a chi-square test (sex) and Mann-Whitney *U* test (age and QTc interval). Genotype frequencies were tested for Hardy-Weinberg equilibrium using the chi-square test with 1 degree of freedom. The genotype frequencies, assuming an

additive model, were compared between the whole group of case subjects and control subjects, between subjects on amiodarone and control subjects, and between subjects on sotalol and control subjects (case-control analysis) using logistic regression analysis in PLINK (version 1.07) (21,22). Age and sex were not used as covariates because the sex distribution appeared not to be significantly different between case subjects and control subjects, and control subjects appeared to be older than case subjects were; hence, they were more likely to develop QT prolongation or ventricular arrhythmia but, nevertheless, did not demonstrate either. As QTc interval appeared to be significantly longer among the case subjects after removal of the drug than among the control subjects, we also tested the model where QTc interval was included to correct for possible mediating effects. Case subjects who had a ventricular- or atrioventricular-paced ECG or a left branch bundle block were excluded from this analysis (n = 12). The same analysis was performed in the replication study for our top SNP rs10919035.

In addition, we studied QTc interval as a quantitative trait in the population-based DARE control sample (74 of 87 controls had data on QTc interval available) in order to replicate published associations. Case subjects were not included in this analysis as they had a longer QTc interval even after removal of the drug and hence were not representative of the population. A linear regression was performed for each SNP following an additive model on QTc interval with SNP, age, and sex as covariates. A similar analysis was done in the BRIGHT cohort (n = 1,480) and the results of the 2 cohorts were meta-analyzed using the fixed-effect inverse variance method in PLINK (version 1.07) (21,22).

Because multiple SNP were tested, a multiple testing correction was applied using SNP Spectral Decomposition (26,27). This method calculates the effective number of independent marker loci accounting for linkage disequilibrium between the SNP. With this number, a Bonferroni correction is applied to assess the significance threshold. For our dataset of 167 SNP in the *NOS1AP* gene, the effective number was 98.8, and hence a p value less than 0.00052 was considered statistically significant. Odds ratios (OR) and 99.95% confidence intervals (CI) were calculated to assess the strength of the association. Because only 1 SNP was genotyped in the replication study for amiodarone-induced

Table 1 Characteristics of Case Subjects and Control Subjects Included in the Analyses

	DARE Case Subjects	DARE Control Subjects	Vanderbilt Case Subjects	Vanderbilt Healthy Control Subjects	Vanderbilt Control Subjects	BRIGHT
n	58	87	28	68	105	1,480
Age, yrs	62.5 ± 15.5	71.1 ± 10.5*	64 ± 15.16	26.8 ± 5.61	62.2 ± 14.26	57.7 ± 10.18
Female	39 (67)	46 (56)	21 (75)	37 (53)	38 (36)	916 (61.9)
Culpable drug exposure†						
Amiodarone	27 (47)	—	28 (100)	—	—	—
Sotalol	15 (26)	—	—	—	—	—
Diuretics	6 (11)	—	9 (32)	0	43 (41)	
>1 drug	16 (28)	—	0	0	0	
Presentation						
Documented torsades de pointes	50 (89)	—	28 (100)	NA	0	
Ventricular fibrillation/cardiac arrest	12 (21)	—	0	NA	0	
Syncope only	2 (4)	—	0	NA	0	
Hypokalemia	11 (20)	—	7 (25)	NA	0	
Other medical history						
Prior myocardial infarction	14 (25)	—	3 (10)	NA	14 (14)	0 (0)
Heart failure‡	12 (21)	0 (0)	9 (32)	NA	1 (.009)	0 (0)
Atrial fibrillation and/or flutter	29 (52)	—	18 (64)	NA	97 (92)	0 (0)
Congenital long QT syndrome*	2 (4)	0 (0)	0	NA	0	0 (0)
Hypertension	32 (57)	—	13 (46)	NA	65 (62)	1,480 (100)
Hypothyroidism	11 (20)	—	0	NA	18 (17)	0 (0)
Diabetes mellitus	14 (25)	—	0	NA	20 (19)	0 (0)
Maximal QTc during drug exposure, ms				434	467	—
Mean ± SD	592 ± 73.3		584	375–492	403–556	
Range	466–850	—	523–840	428	464	
Median	590		613			
QTc without drug exposures, ms‡						
Mean ± SD	441 ± 25.9	426 ± 18.2*	430	388	443	417 ± 12.0
Range	381–503	379–469	344–505	367–457	367–565	343–704
Median	435	423	428	406	442	420

Values are mean ± SD or n (%) unless otherwise indicated. Dashes indicate that data are not available. *$p<0.05$. †Available for 57 case subjects. ‡From questionnaire data only. §Available for 44 case subjects and 74 control subjects.

BRIGHT = British Genetics of Hypertension Study; DARE = Drug-Induced Arrhythmia Risk Evaluation; NA = not applicable; QTc = corrected QT interval.

QTc interval, a p value <0.05 was considered significant in this cohort.

Results

Study characteristics. Fifty-eight subjects experiencing drug-induced QT prolongation and ventricular arrhythmias (50 [89%] with documented TdP), and 87 healthy control subjects from the DARE study (Table 1) were available for the primary analysis. Control subjects were on average almost 9 years older than case subjects were ($p < 0.001$). No sex difference was observed. Twenty-seven (46%) cases were treated with amiodarone and 15 (27%) with sotalol, whereas 15 (27%) had received more than 1 culpable drug. Eleven cases (20%) were associated with hypokalemia. The cases also had a higher frequency of accompanying structural heart disease and, when the drug exposure was removed, demonstrated greater QTc prolongation than the control subjects did ($p = 1.0 \times 10^{-3}$).

DNA sequencing and association analysis. Sequencing of the *NOS1AP* exonic regions and intron/exon boundaries on chromosome 1 did not identify any novel coding mutations or polymorphisms. However, resequencing 2 kb upstream of the ATG site identified 2 additional SNP that were not included on the Human_CVD 50K Bead Array and these were included in the association analysis.

SNP rs10800397 was significantly associated with drug-induced ventricular arrhythmia and QT prolongation (OR: 3.3, 99.95% CI: 1.0 to 10.8, $p = 3.7 \times 10^{-4}$) (Fig. 1, Table 2). This association was driven by the group of amiodarone users (rs10800397: OR: 4.5, 99.95% CI: 1.0 to 19.8, $p = 4.3 \times 10^{-4}$; case subjects: 37.0%, control subjects: 14.4%) (Fig. 1, Table 2). For this subgroup of cases, 3 noncoding SNPs were significantly associated with drug-induced ventricular arrhythmia and QT-interval prolongation (most significant SNP rs10919035: OR: 5.5, 99.95% CI: 1.1 to 27.9, $p = 3.0 \times 10^{4}$; allele frequencies: case subjects: 27.8%, control subjects: 7.1%). These SNPs did not include either of the SNP rs10494366 (OR: 1.1, $p = 0.79$; allele frequencies amiodarone-induced: case subjects: 37.0%, control subjects: 35.1%) and rs16857031 (OR: 1.8, $p = 0.13$; allele frequencies amiodarone-induced: case subjects: 27.8%, control subjects: 19.0%) known to be

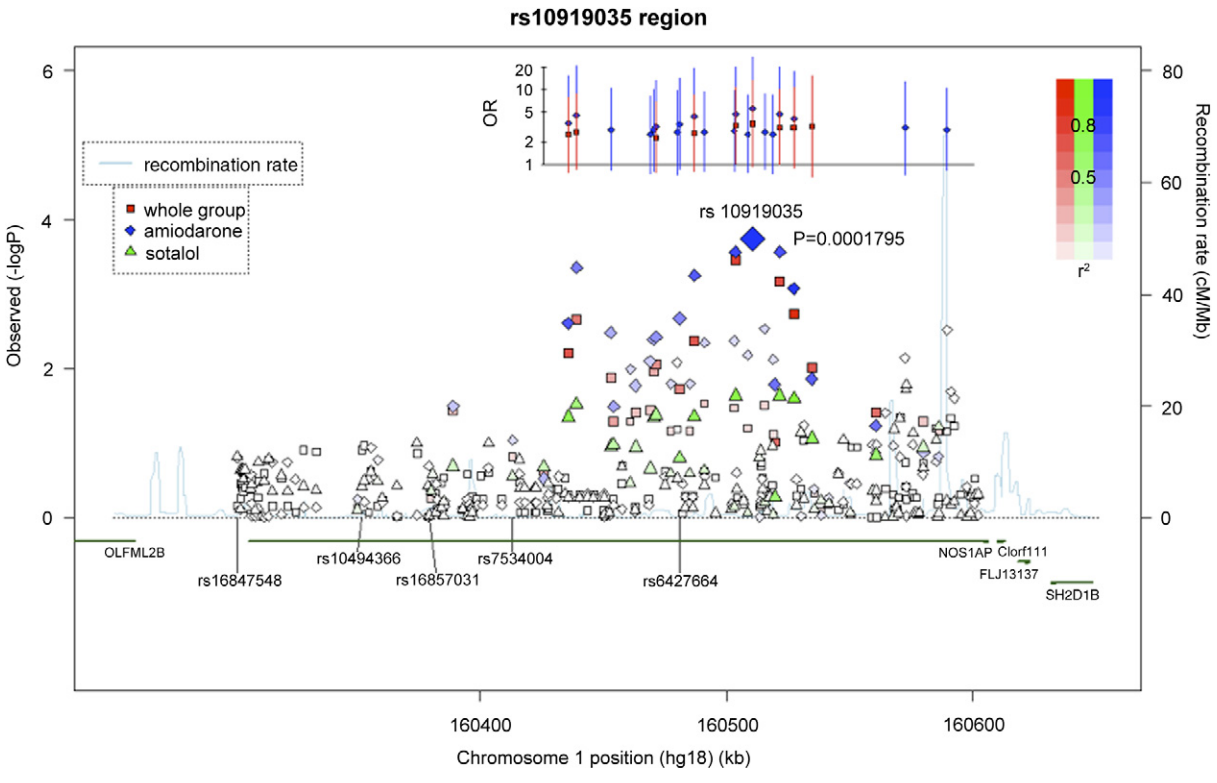

Figure 1 **Regional Association Plot for *NOS1AP* SNPs**

Statistical significance of single nucleotide polymorphisms (SNPs) are shown on the –log (p) scale for the whole group **(red squares),** the amiodarone-users **(blue diamonds),** and the sotalol users **(green triangles).** The recombination rate is shown at the right axis. Odds ratios (OR) and 99.95% confidence intervals (CI) of the top SNPs ($p < 0.01$) are shown in the top of the figure (whole group in **red;** amiodarone-users in **blue**). The most significantly associated SNP is represented by a **large blue diamond.** The correlation of this SNP to other SNPs at the *NOS1AP* locus is shown on a scale from minimal **(white)** to maximal **(bright red/green/blue).** The rs-ids of SNPs previously associated with corrected QT-interval prolongation in the general population or that where in strong linkage disequilibrium with such SNPs are given in the lower part of the figure. Figure made using an adapted version of the R script of SNAP (Johnson AD. SNAP: a web-based tool for identification and annotation of proxy SNPs using HapMap. Bioinformatics 2008;24:2938–9).

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Table 2 Top Drug-Induced Arrhythmia Associated SNPs ($p < 0.01$) in the Group of 27 Amiodarone Users, the Group of 15 Sotalol Users, and the Whole Group of 58 Case Subjects Compared With 87 Control Subjects

SNP	Minor Allele	Control Subjects MAF (%)	Amiodarone Users				Sotalol Users				All Cases			
			AF (%)	p Value	OR	99.95% CI	AF (%)	p Value	OR	99.95% CI	AF (%)	p Value	OR	99.95% CI
rs10919035	T	7.1	27.8	**3.0×10^{-4}**	5.5	1.1–27.9	20.0	0.022	4.1	0.5–34.2	20.7	0.0011	3.7	0.9–14.6
rs10800397	T	14.4	37.0	**4.3×10^{-4}**	4.5	1.0–19.8	30.0	0.023	3.7	0.5–27.8	31.0	**3.7×10^{-4}**	3.3	1.0–10.8
rs10800404	T	14.4	37.0	**4.3×10^{-4}**	4.5	1.0–19.8	30.0	0.023	3.7	0.5–27.8	30.2	6.9×10^{-4}	3.1	1.0–10.1
rs10800352	G	14.4	35.2	7.1×10^{-4}	4.4	1.0–20.2	30.0	0.030	3.2	0.5–20.4	28.5	0.0023	2.8	0.9–8.8
rs7522678	A	13.2	33.3	9.0×10^{-4}	4.2	0.9–19.0	26.7	0.043	3.2	0.4–23.3	25.9	0.0042	2.7	0.8–8.8
rs10800409	T	8.6	27.8	0.0013	4.0	0.9–17.6	23.3	0.023	3.5	0.5–23.3	22.4	0.0016	3.2	0.9–11.3
rs6427664	A	19.0	38.9	0.0025	3.4	0.8–14.0	30.0	0.14	2.1	0.4–11.9	31.0	0.015	2.1	0.7–5.9
rs10918859	A	12.6	29.6	0.0039	3.4	0.8–14.8	26.7	0.044	2.9	0.5–18.5	25.0	0.0061	2.5	0.8–8.1
rs12403202	T	20.7	40.7	0.0045	2.8	0.8–10.0	16.7	0.59	0.7	0.1–5.1	29.8	0.077	1.7	0.6–4.5
rs12742393	A	36.1	57.4	0.0053	2.7	0.8–9.4	46.7	0.24	1.7	0.4–7.7	48.3	0.032	1.8	0.7–4.4
rs6664702	C	17.8	35.2	0.0058	3.1	0.7–12.8	33.3	0.043	2.8	0.5–16.1	30.2	0.0094	2.3	0.8–6.9
rs10753784	G	35.6	57.4	0.0061	2.5	0.8–8.1	40.0	0.64	1.2	0.3–5.2	48.3	0.034	1.7	0.7–4.1
rs4298709	G	39.1	59.3	0.0077	2.6	0.7–9.1	42.9	0.69	1.2	0.3–5.5	51.8	0.031	1.7	0.7–4.3
rs4531275	T	25.9	44.4	0.0085	2.7	0.7–9.6	39.3	0.12	2.1	0.4–11.1	38.6	0.016	2.0	0.7–5.4
rs10399680	C	21.3	38.9	0.0098	2.6	0.7–9.5	30.0	0.28	1.7	0.3–8.3	31.0	0.058	1.7	0.6–4.6

SNPs are sorted according to significance in the analysis of amiodarone users. Significant SNPs ($p < 0.00052$) are denoted in **bold**.
CI = confidence interval; (M)AF = (minor) allele frequency; OR = odds ratio; SNP = single nucleotide polymorphism.

associated with variation in resting QTc nor SNPs that were in strong linkage disequilibrium with them (9,11,12). The remaining *NOS1AP* SNPs previously associated with resting QTc (rs12143842, rs12029454, rs4657178) were not included on the array and hence could not be investigated directly. However, SNPs that were in strong linkage disequilibrium (LD) with these in the HapMap (Haplotype Map) Project's western and northern European ancestry (CEU) population (26) and that were present on the chip were not associated with amiodarone-induced ventricular arrhythmia and QT interval prolongation, except for SNP rs6427664, which was in LD with rs4657178 ($r^2 = 0.82$ in HapMap CEU sample) and showed suggestive association (OR: 3.4, 99.95% CI: 0.8 to 14.0, $p = 0.0025$). Including QTc interval as a covariate in the model weakened the associations, but the ORs did not change (Table 3). None of the SNP was statistically significantly associated with sotalol-induced ventricular arrhythmia and QT-interval prolongation, although the top SNPs were the same as for amiodarone (Tables 2 and 3).

The most significant SNP (rs10919035) was also genotyped in the replication cohort of 28 cases with amiodarone-induced LQTS and 173 drug-challenged control subjects. The effect of this SNP showed a trend in the same direction as that observed in the DARE cohort (allele T: 26.8% among case subjects versus 16.5% in drug-challenged control subjects; $p = 0.060$). Meta-analysis of the results of rs10919035 from both studies revealed an OR of 2.81 for each T allele (99.95%: CI 1.62 to 4.89, $p = 2.4 \times 10^{-4}$).

To validate the reported association between *NOS1AP* and QTc interval duration, the 167 SNP spanning the *NOS1AP* gene were examined for association in the population-based DARE control subjects (n = 74). None of the SNPs was significantly associated with QT interval after multiple testing correction (Table 4, Fig. 2A). To extend this analysis, we combined the data in a meta-analysis with those of the BRIGHT cohort (n = 1,480) (Table 4, Fig. 2A). The results of the BRIGHT cohort were similar to those of the DARE cohort and, although the associations were more significant, the effect sizes were 2× to 4× smaller. SNP rs12734991 showed the largest increase in QTc interval in the DARE cohort ($\beta = 11.1$ ms per C allele, $p = 6.4 \times 10^{-4}$), and it was second in the meta-analysis ($\beta = 4.2$ ms per C allele, $p = 3.5 \times 10^{-6}$). The most associated SNPs overlap with those SNPs arising from the case-control analysis. SNP rs10800397, which showed the strongest association with drug-induced ventricular arrhythmia and QT-interval prolongation, also caused an increase in QTc interval in the control subjects (in DARE/meta-analysis: $\beta = 11.5/4.6$ ms per T allele, $p = 0.019/4.3 \times 10^{-5}$). In addition, SNP rs6427664, which was in LD with rs4657178, reached a significance level of $0.012/9.1 \times 10^{-5}$ in DARE/meta-analysis ($\beta = 11.1/4.2$ ms per A allele). In contrast, SNP rs10494366 and rs16857031 were not significant ($p = 0.85/0.005$ and 0.95/22, respectively, in DARE/

Table 3 Top Drug-Induced Arrhythmia Associated SNPs ($p < 0.01$) Corrected for QTc Interval in the Group of 19 Amiodarone Users, the Group of 13 Sotalol Users, and the Whole Group of 44 Case Subjects Compared With 74 Control Subjects

		Amiodarone Users			Sotalol Users			All Cases		
SNP	Minor Allele	p Value	OR	99.95% CI	p Value	OR	99.95% CI	p Value	OR	99.95% CI
rs10919035	T	*0.0023*	6.6	0.8–56.7	0.14	2.9	0.2–37.2	0.011	3.5	0.6–19.3
rs10800397	T	0.066	2.8	0.4–20.7	0.13	2.7	0.3–27.6	0.014	2.8	0.7–11.9
rs10800404	T	0.066	2.8	0.4–20.7	0.13	2.7	0.3–27.6	0.024	2.6	0.6–10.9
rs10800352	G	*0.0098*	4.5	0.6–34.8	0.035	3.7	0.4–31.8	0.014	2.7	0.7–10.7
rs7522678	A	0.016	4.0	0.5–29.5	0.21	2.3	0.2–23.2	0.069	2.2	0.5–9.3
rs10800409	T	0.013	4.0	0.6–27.1	0.10	2.8	0.3–23.7	0.027	2.7	0.6–12.3
rs6427664	A	0.21	1.9	0.3–12.0	0.51	1.5	0.2–10.7	0.22	1.6	0.4–5.6
rs10918859	A	0.036	3.3	0.5–23.6	0.046	3.4	0.4–28.3	0.028	2.4	0.6–10.0
rs12403202	T	0.044	2.5	0.5–12.5	0.51	0.7	0.1–5.9	0.37	1.4	0.4–4.4
rs12742393	A	0.74	1.2	0.3–5.3	0.33	1.6	0.3–8.7	0.23	1.5	0.5–4.5
rs6664702	C	0.078	2.6	0.4–16.2	0.18	2.2	0.3–16.3	0.12	1.8	0.5–6.8
rs10753784	G	0.31	1.6	0.3–7.2	0.82	0.9	0.2–5.2	0.42	1.3	0.4–3.8
rs4298709	G	0.55	1.3	0.3–6.8	0.71	0.8	0.1–5.6	0.34	1.4	0.4–4.1
rs4531275	T	0.089	2.3	0.4–11.9	0.18	2.2	0.3–15.8	0.079	1.9	0.5–6.3
rs10399680	C	0.47	1.4	0.3–7.1	0.89	1.1	0.2–6.9	0.56	1.2	0.4–4.1

SNPs are sorted in the same order as in Table 2. Significant SNPs ($p < 5.2 \times 10^{-4}$) are denoted in bold. Other p values <0.01 are shown in italic.
Abbreviations as in Tables 1 and 2.

meta-analysis), and neither were SNPs that were in strong LD with other previously QTc-associated nontyped SNPs rs12143842 and rs12029454.

Discussion

This study is the first to demonstrate that common variations in the *NOS1AP* gene are associated with a significant increase in the risk of drug-induced, and in particular amiodarone-induced, ventricular arrhythmia and QT prolongation. Prolongation of the QT interval associated with TdP is currently the most common cause of withdrawal or restriction of the use of antiarrhythmic drugs (29).

We performed a comprehensive screen of 167 SNPs in and close to the *NOS1AP* gene in order to investigate association of *NOS1AP* variations with drug-induced ventricular arrhythmia and prolongation of the QT interval. SNP rs10800397 reached a significance level of $p = 3.7 \times 10^{-4}$ in the overall DARE case-control study and this was predominantly explained by the subgroup of amiodarone users ($p = 4.3 \times 10^{-4}$). For this subgroup of cases, 3 SNPs were significantly associated with drug-induced ventricular arrhythmia and QT-interval prolongation. The most significant one, rs10919035, was in moderate LD with rs10800397 ($r^2 = 0.49$). We carried out a replication study using amiodarone-treated cases from a second study (Vanderbilt) and these were compared with a drug-challenged control group. Although we could not fully validate our results from the DARE cases and nondrug-challenged control subjects, meta-analysis of the results of rs10919035 from both studies revealed an OR of 2.81 for each T allele (99.95% CI: 1.62 to 4.89, $p = 2.4 \times 10^{-4}$). Interestingly, the allele frequency among the replication and DARE cases was identical. The nonsignificance in the replication study appears to be caused by the unexpectedly higher frequency among the control subjects (17% versus 7% in DARE control subjects, 8% in 1000Genomes CEU, and 11% in HapMap Phase 2 CEU (28)) and might therefore be a population-specific effect. It is also important to note that some of the drug-challenged control subjects were challenged with ibutilide. Unlike amiodarone, ibutilide does not produce its prolongation of action potential via inhibition of potassium channels including I_{Kr}, nor does it have a sodium-blocking, antiadrenergic, and calcium-blocking activity.

In contrast, ventricular arrhythmia and QT prolongation induced by sotalol were not significantly affected by *NOS1AP*, although the same SNPs demonstrated the largest genotype differences with smaller ORs. This subgroup was too small (n = 15) to draw any certain conclusions about drug-specific interactions.

A common pathway between amiodarone-induced LQTS and *NOS1AP* common variation could be the role of the NOS regulator pathway in cardiac L-type Ca_V currents. In the prospective population-based Rotterdam Study, van Noord et al. (28) associated minor alleles of the *NOS1AP* SNP rs10494366 and rs10918594 with the modification of the QTc prolonging effect of verapamil. Furthermore, Chang et al. (16) found that overexpression of the *NOS1AP* gene product in isolated guinea pig myocytes causes attenuation of the L-type Ca_V current, a slight increase in rapid delayed rectifier current (I_{Kr}), and shortening of action potentials.

Data from the congenital LQTS may also support such a mechanism. Rare variants in the LQT8 gene *CACNA1C*, encoding a subunit of the L-type Ca_V channel, cause an unusual form of LQTS by increasing calcium influx into the myocyte (17,18). The recently identified LQT12 gene,

Table 4 Top SNPs From the DARE Cohort ($p < 0.05$) Associated With the Continuous QTc Interval in 74 Control Subjects Ordered According to Their Significance Together With the Results of the BRIGHT Cohort (n = 1,480) and of the Meta-Analysis of the 2 Cohorts

			DARE			BRIGHT			Meta-Analysis		
SNP	Position	Allele	Beta	SE	p Value	Beta	SE	p Value	Beta	SE	p Value
rs12734991	160461200	C	11.1	3.1	6.4×10^{-4}	3.5	0.9	$\mathbf{1.7 \times 10^{-4}}$	4.2	0.9	$\mathbf{3.5 \times 10^{-6}}$
rs4657166	160427963	G	11.5	3.6	*0.0023*	2.6	1.0	0.010	3.2	1.0	*0.00091*
rs12733377	160519067	G	9.3	3.1	*0.0037*	3.3	0.9	$\mathbf{4.0 \times 10^{-4}}$	3.8	0.9	$\mathbf{2.0 \times 10^{-5}}$
rs12729882	160508661	A	8.9	3.0	*0.0049*	3.1	0.9	8.6×10^{-4}	3.6	0.9	$\mathbf{5.2 \times 10^{-5}}$
rs2661818	160531438	G	8.6	3.0	*0.0051*	4.0	0.9	$\mathbf{1.7 \times 10^{-5}}$	4.4	0.9	$\mathbf{6.1 \times 10^{-7}}$
rs10399680	160480119	C	11.1	3.8	*0.0052*	3.7	1.1	7.9×10^{-4}	4.2	1.0	$\mathbf{5.7 \times 10^{-5}}$
rs6664702	160471531	C	11.7	4.4	*0.0099*	3.6	1.1	*0.0013*	4.1	1.1	$\mathbf{1.6 \times 10^{-4}}$
rs1964052	160602048	T	11.6	4.4	0.011	−1.1	1.4	0.44	0.1	1.3	0.95
rs4298709	160503206	G	8.5	3.3	0.011	3.0	0.9	*0.0015*	3.4	0.9	$\mathbf{1.6 \times 10^{-4}}$
rs10918936	160469112	A	8.1	3.1	0.011	2.9	1.0	*0.0025*	3.3	0.9	$\mathbf{2.5 \times 10^{-4}}$
rs6427664	160481097	A	11.1	4.3	0.012	3.7	1.1	7.6×10^{-4}	4.2	1.1	$\mathbf{9.1 \times 10^{-5}}$
rs905720	160600724	T	8.5	3.5	0.018	−0.4	1.0	0.73	0.4	1.0	0.72
rs4557949	160477578	A	8.0	3.3	0.019	3.8	0.9	$\mathbf{4.9 \times 10^{-5}}$	4.1	0.9	$\mathbf{4.8 \times 10^{-6}}$
rs4145621	160485312	C	8.0	3.3	0.019	3.9	0.9	$\mathbf{3.7 \times 10^{-5}}$	4.2	0.9	$\mathbf{3.5 \times 10^{-6}}$
rs10800397	160503714	T	11.5	4.8	0.019	4.2	1.1	$\mathbf{3.0 \times 10^{-5}}$	4.6	1.1	$\mathbf{4.3 \times 10^{-5}}$
rs10800404	160521736	T	11.5	4.8	0.019	4.4	1.2	$\mathbf{1.4 \times 10^{-4}}$	4.8	1.1	$\mathbf{1.9 \times 10^{-5}}$
rs12135795	160416389	A	7.6	3.2	0.019	2.3	0.9	0.016	2.7	0.9	*0.0026*
rs10753765	160418397	G	7.6	3.2	0.019	2.3	0.9	0.016	2.7	0.9	*0.0026*
rs3927640	160422437	T	7.6	3.2	0.019	2.3	0.9	0.015	2.7	0.9	*0.0025*
rs4657161	160422913	G	7.6	3.2	0.019	2.3	0.9	0.015	2.7	0.9	*0.0025*
rs12090585	160305400	A	−8.9	3.9	0.024	NA	NA	NA	NA	NA	NA
rs347271	160585953	A	19.6	8.7	0.027	2.4	2.5	0.34	3.7	2.4	0.12
rs4328057	160458029	G	19.7	8.8	0.028	−1.8	3.1	0.57	0.6	2.9	0.84
rs10918951	160475138	A	11.6	5.4	0.036	0.2	2.1	0.91	1.7	2.0	0.38
rs16859092	160491174	C	11.6	5.4	0.036	−0.2	2.1	0.92	1.3	2.0	0.50
rs12742393	160491210	A	6.9	3.3	0.039	3.2	0.9	7.5×10^{-4}	3.5	0.9	$\mathbf{1.3 \times 10^{-4}}$
rs4656364	160545479	C	10.0	4.8	0.040	3.3	1.5	0.033	3.9	1.5	*0.0076*
rs10753784	160515544	G	6.6	3.2	0.043	3.3	1.0	5.5×10^{-4}	3.6	0.9	$\mathbf{9.4 \times 10^{-5}}$
rs10919035	160510636	T	14.1	6.9	0.043	5.1	1.4	$\mathbf{3.3 \times 10^{-4}}$	5.5	1.4	$\mathbf{8.1 \times 10^{-5}}$

Significantly associated SNPs are shown in **bold** ($p < 5.2 \times 10^{-4}$). p values <0.01 are shown in *italic*.
SE = standard error; other abbreviations as in Tables 1 and 2.

alpha-1-syntrophin (SNTA1), has also been shown to be involved in the nNOS pathway (19). A mutation in the gene causes inhibition of nNOS and is associated with increased peak and late sodium currents. Therefore, genes encoding proteins interacting with nNOS have the potential to alter cardiac repolarization, perhaps by influencing calcium cycling in cardiac myocytes. *NOS1AP* minor allele variants have more recently been associated with modification of the severity of presentation of LQTS (31,32) and the risk of sudden death in coronary artery disease (33).

In addition, we studied QT interval as a quantitative trait in population-based control subjects. In the meta-analysis of the DARE control subjects (n = 74) and the BRIGHT cohort (n = 1,480), 22 SNPs reached significance ($p < 5.2 \times 10^{-4}$). Although the results in the BRIGHT cohort were more significant as a result of the larger sample size, the effects of the SNPs were 2× to 4× smaller than in the DARE control subjects. Furthermore, many of the top hits of this analysis overlap with the top hits of the drug-induced case-control analysis. This implies that the effect of *NOS1AP* on drug-induced LQTS is not independent of the effect of *NOS1AP* on QT interval in general. Because case subjects and control subjects already demonstrated significantly different mean QTc intervals after drug removal, we also corrected the case-control drug-induced LQTS interval analysis for baseline QTc interval. Although the results became less significant, the ORs only diminished slightly. This suggests that the investigated QT-interval prolonging drugs and in particular amiodarone interact with *NOS1AP* variants. Unfortunately, our study design does not allow testing for interactions directly as all case subjects used drugs, whereas none of the DARE control subjects did and the drug-challenged control subjects from the Vanderbilt study were not on amiodarone specifically. The findings do lend support, however, to repolarization reserve being influenced by common genetic variation.

Another explanation for the larger QTc interval among case subjects after removal of the drug may be that the case subjects demonstrated a higher frequency of hypertension and underlying cardiac disease than the healthy control

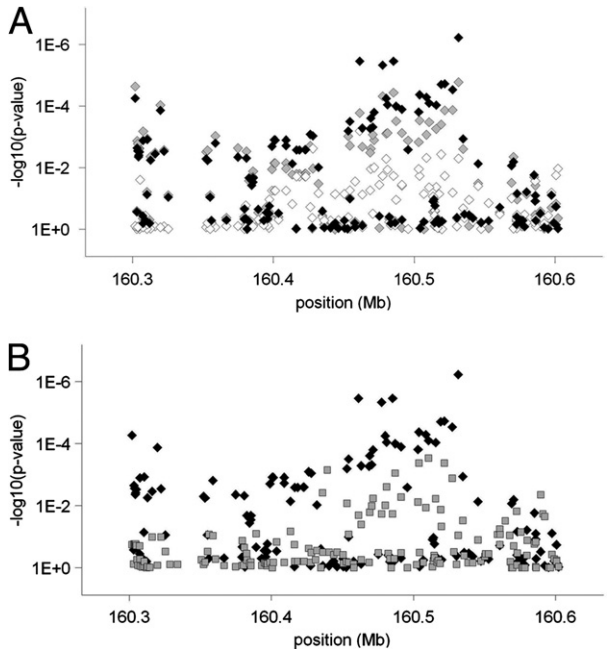

Figure 2 **Association of the *NOS1AP* SNPs With the Continuous QTc Interval**

(A) Association of the *NOS1AP* single nucleotide polymorphisms (SNP) with the continuous corrected QT (QTc) interval in the DARE (Drug-Induced Arrhythmia Risk Evaluation) study control subjects **(white diamonds)** (n = 74), in the BRIGHT cohort **(gray diamonds)** (n = 1,480), and in the meta-analysis of the 2 cohorts **(black diamonds)** (n = 1,554) and **(B)** association of the *NOS1AP* SNP with the continuous QTc interval in the DARE-BRIGHT meta-analysis **(black diamonds)** (n = 1,554) and with dichotomous drug-induced ventricular arrhythmia and QT-interval prolongation **(gray squares)** (n = 58 case subjects and 87 control subjects).

subjects did. These are known acquired risk factors for QT-interval prolongation and TdP (34).

There are currently no published functional studies investigating variable expression of *NOS1AP* polymorphisms. Given the shared effects of *NOS1AP* and amiodarone on L-type calcium and potassium currents, however, one might hypothesize that individuals carrying genetic variants in *NOS1AP,* which impair its expression, and in turn, result in increased L-type calcium currents and/or QT prolongation, may have additional arrhythmogenic risk with amiodarone therapy. These common variants may have relevance for future pharmacogenomic applications in clinical practice permitting safer prescription of amiodarone for vulnerable patients. Development of safer novel drugs may also benefit from this improved understanding of the biological pathways underlying the variation in drug response.

Study strengths. First, the DARE case subjects were collected prospectively and nationally in a systematic manner with identical comprehensive phenotyping, a strength compared with other series. Second, the coverage of the *NOS1AP* gene was comprehensive and included 167 polymorphic SNPs in and close to the *NOS1AP* gene. Third, the high ORs and level of significance despite small numbers provides compelling support for the association as does the similar frequency of SNPs in cases in the replication case-control study and the trend toward a significant association.

Study limitations. First, we were limited by the rarity of subjects treated with amiodarone that presented with ventricular arrhythmia and QT prolongation. Second, whereas the DARE control subjects were originally matched for age, sex, and ethnicity, it was difficult to also match for drug exposure and other comorbidity, which results in difficulties in determining whether the associations identified are caused by drug exposure or by the underlying arrhythmic event. The replication case-control study was however able to use controls exposed to QT-prolonging drugs although not amiodarone specifically. Third, as with previous resequencing efforts of *NOS1AP* (9), we did not identify any missense mutations that explain the association results. The associated *NOS1AP* SNPs are therefore not functional variants and are only in linkage disequilibrium with the causal SNP or regulatory DNA element.

Conclusions

Our study shows that common variants in the *NOS1AP* gene play a role in the pathogenesis of drug-induced, and particularly amiodarone-induced, LQTS. These variants may be used in the future as markers to predict and avoid risk for drug-induced TdP in Caucasian patients who may require amiodarone therapy.

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Reprint requests and correspondence: Dr. Yalda Jamshidi, Senior Lecturer in Human Genetics, Genetics Research Centre, St. George's University of London, Cranmer Terrace, London SW17 0RE, United Kingdom. E-mail: y.jamshidi@sgul.ac.uk. OR Dr. Elijah R. Behr, Cardiovascular Sciences Research Centre, St. George's University of London, Cranmer Terrace, London SW17 0RE, United Kingdom. E-mail: e.behr@sgul.ac.uk.

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Key Words: cardiac arrhythmias ■ drug ■ genetics ■ *NOS1AP* ■ risk stratification ■ single nucleotide polymorphisms.